Observations on the Gametogenesis of Grantia compressa.

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With Plates 23 to 26.

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(A) General Introduction.

The problem of the gametogenesis of Sponges is one which has attracted a good deal of attention from time to time, but it can hardly be said that our knowledge of the subject is as yet by any means in a satisfactory condition. As far back as 1851 Huxley described the occurrence of supposed spermatozoa in Tethya, and in 1854 Carter did the same for Spongilla, but Haeckel is probably right in supposing that both these authors were in reality describing flagellate collared cells, a mistake which, owing to the difficulty of detecting the collar in certain conditions, it is very easy to make. Haeckel, indeed, claims for Lieberkühn the original discovery in 1856 of the sexual differentiation of sponges and of the ova and spermatozoa (in Spongilla).

Haeckel points out the great difficulty in finding the spermatozoa of sponges, a difficulty which has been, and doubtless still is, experienced by many spongologists, and which arises chiefly, no doubt, from their extremely minute size, the fact that they occur scattered throughout the sponge and not segregated in definite gonads, and their liability to confusion with other tissue elements. The ova, on the other hand, in their later stages of growth, are readily recognisable by their large size, and, at certain periods, their conspicuous vesicular nuclei, although these again are not collected in definite ovaries, but occur scattered through the sponge-tissues.

The spermatozoa of calcareous sponges were discovered by Haeckel himself in 1871, and at about the same time by Eimer. In his well-known monograph, "Die Kalkschwämme" (1872) the former author gives an account of all that was previously known of the germ-cells of sponges, and describes his own observations and conclusions with regard to the Calcarea. He finds reasons for deriving the spermatozoa from collared cells of the so-called entoderm (gastral layer), and gives several figures of sperm-morulæ lying amongst the ordinary collared cells in the gastral layer of several species.

With regard to the ova he remarks that the question of the origin and of the original position of the egg-cells is the most difficult and darkest part in the histology of calcareous sponges, an opinion with which those who have studied the question will hardly feel inclined to quarrel. Haeckel himself at first regarded the ova as originating in the "exoderm," in which he, of course, includes everything but the "entoderm," but his more mature conclusion is that they really originate in the "entoderm." He says (1872, p. 159): "Einzelne Geisselzellen des Entoderms vergrössern sich, ziehen ihren schwingenden Geisselfortsatz ein, und entwickeln sich direct durch Aufblähung des Kernes und bedeutende Volums-Zunahme des Protoplasma zu Eizellen."

Since Haeckel wrote, opinion as to the origin of the germ-cells in sponges has changed, and though very few writers have recorded any detailed observations, it is generally held, in accordance with the teachings of F. E. Schulze and others, that both ova and spermatozoa arise from amœboid wandering cells in the mesoglœa, which, of course, is part of Haeckel's "exoderm." No one, since Haeckel's time, appears to have seen the sperm-morulæ lying amongst the collared cells in the walls of the flagellate chambers, and no one has traced the stages by which collared cells might be supposed to have been converted into spermatogonia. Haeckel himself seems to have been by no means satisfied that his own observations on the subject were conclusive, and Poléjaeff, writing ten years later (1882), evidently regarded them with grave suspicion.

Nevertheless, I believe that Haeckel was essentially right in maintaining that oögonia and spermatogonia both arise from collared cells. It is, at any rate, quite certain that bodies resembling spermatogonia are frequently to be found in small morula-like clusters amongst the collared cells in the walls of the flagellate chambers of Grantia compressa, very much as figured by Haeckel for other Calcarea, except that I have never observed the tails of the spermatozoa in this situation, and, indeed, have only seen the sperm-morulæ

in properly prepared, stained sections. Between the original collared cell and the mature germ-cell of either sex amœboid stages intervene, which are frequently to be found in the mesoglœa, at any rate in the case of the oögonia. It is an easy matter to trace the growth of the amœboid oögonia into mature ova, but the question is—Whence come the earliest amœboid stages ?

The modern answer to this question is that they are derived from primitive amœboid cells ("archæocytes"), but I cannot help suspecting that this answer is merely an echo of Weismann's well-known views as to the early segregation of the germ-cells and the continuity of the germ-plasm. It depends, so far as I am aware, not upon direct observation, but upon a process of reasoning by exclusion. The germ-cells are supposed to remain over after the somatic cells have been subtracted from the sum total of cells derived from the segmenting ovum.

I believe that this particular theory of the origin of the germ-cells in sponges originated with Dr. Otto Maas (1894), who sums up his conclusions as follows (p. 35) :

" (1) Wir können hier eine directe Abstammung der Keimzellen der einen Generation vom Ei nachweisen, indem durch Subtraction aller somatischen specialisierten Elemente schliesslich eine Anzahl indifferent gebliebener Elemente übrig ist, die Urgeschlechtszellen.

" (2) Der Hauptunterschied zwischen den somatischen und den Geschlechtszellen zeigt sich vom Anfang wie später im Kern, und zwar in der Quantität und Anordnung des Chromatins."

Anyone who has studied the gametogenesis of sponges knows how greatly the condition of the chromatin varies in different stages, and I do not believe it is possible to indicate any nuclear character by which primordial germ-cells can be definitely distinguished from somatic cells, at any rate in the present state of our knowledge. The alleged distinction between the two groups of cells is a purely theoretical one. Indeed, the latest writer on the subject, Max Jörgensen (1910), has already come to the conclusion that the distinction which Maas endeavours

to draw between germ-cells and somatic cells cannot be maintained in sponges, and "dass es sehr wohl zur Bildung von Geschlechtszellen aus somatischen Zellen kommen kann, wie man dies ja bei dem primitiven Charakter der Schwämme von vornherein erwarten würde" (p. 170). This author derives the oögonia of Sycon from ordinary "mesoderm" cells rather than from primitive undifferentiated amœbocytes (archæocytes), though he thinks that the latter may also give rise to oögonia. He does not deal with the spermatogenesis.

My own attention was first particularly directed towards the problem of the origin of the germ-cells in calcareous sponges under the following circumstances. Some years ago Prof. Herdman invited me to write a memoir on the common British species, Grantia compressa. In the course of this work it became evident that although the ova of this sponge were easily recognisable in the maternal tissues in various stages of growth, and also embryos in various stages of development, no one had ever been able to find the spermatozoa, though they were searched for repeatedly by Mr. Carter (1875, p. 25). For the sake of completeness it appeared highly desirable that this gap in our knowledge should be filled.

In April, 1912, accordingly, I paid a visit to the laboratory of the Marine Biological Association at Plymouth, and although I was unable to find spermatozoa in the living specimens, of which I examined a large number, I preserved material which on subsequent investigation furnished the clue to the solution of the problem. I found that the sponge is hermaphrodite, producing both male and female germ-cells simultaneously, but that the spermatozoa are produced in comparatively small numbers, the minute sperm-morulæ being scattered here and there, enclosed in spermatocysts, between the collared cells of the chamber walls, and also occurring free in the flagellate chambers. The spermatogonia in these sperm-morulæ are extremely minute, and I am unable to say anything with regard to their mode of division. Apparently they are sometimes transferred as sperm-morulæ to the inhalant canals of the

same or of another individual, where they break up into spermatozoa, but it is probable that they may sometimes break up into spermatozoa before leaving the parent sponge. The evidence on these points is, however, curiously scanty, and I conclude that the spermatozoa are rarely, if ever, liberated in large numbers.

With regard to the process of oögenesis, on the other hand, I found a number of very interesting stages, and with the exception of the second maturation spindle (described by Max Jörgensen in Sycon), am able to give a fairly complete account. My observations agree in many respects with those recorded by Jörgensen on the oögenesis of Sycon, but I am able to add a good deal to his account, and in some respects my interpretations are different. Though I have repeatedly observed mitotic figures of various types I have always found the chromosomes very small and ill-defined, and I cannot pretend to give such precise descriptions of the mitotic phenomena as Jörgensen has done. It is very difficult to understand his account of these phenomena, or to harmonise it with currently accepted views, but I must leave to specialists in cytology the detailed criticism of his work in this respect. I may perhaps say, however, that many of his figures appear to me to be very diagrammatic.

One of the most remarkable phenomena observed during my investigations is the nutrition of the growing oöcyte by means of phagocytic nurse-cells. Very extensive phagocytosis has also been observed in the case of certain large amœbocytes, which seem to devour the young germ-cells in some cases in a wholesale fashion.

Though well aware that I am not able to give by any means a complete account of the history of the germ-cells in Grantia compressa, I hope that the following pages may not only help to fill a conspicuous gap in our knowledge of this common British sponge, but also throw some new light on the difficult problem of gametogenesis in sponges generally.

I must take this opportunity of thanking my friends at the Plymouth Laboratory, especially Dr. Allen and Mr. Orton,

for their hospitality and assistance during my visit. I am also greatly indebted to the University of London for the use of their table at the Laboratory.

(B) Material and Methods of Investigation.

My observations at the Plymouth Laboratory, made from April 10th to April 22nd, 1912, were mainly directed towards the discovery of the spermatozoa of Grantia compressa. The sponge may be obtained in large numbers close to the Laboratory, at low water, and I also received a number of fresh specimens from Drake's Island and Rum Bay. They varied greatly in size, from quite small to as much as 80 mm. in height by 18 mm. in breadth (a specimen from Rum Bay). A large number were microscopically examined in the living condition, either by teasing or by means of hand sections, or by pipetting out the contents of the central gastral cavity, but my search for living spermatozoa was fruitless.

I preserved a considerable number of specimens, however, for future examination, and the results recorded in the present paper are based almost entirely upon the study of these by means of paraffin sections.

The material that turned out satisfactorily was fixed either in strong Flemming's solution, or in a mixture of Flemming, formol and sea-water. In the former case it was graded up, after washing, to 70 per cent. alcohol; in the latter it was preserved in formol sea-water.

The sections were, for the most part, cut of a thickness of 5 μ and stained on the slide. I found that iron-brazilin gave excellent results, but iron-hæmatoxylin was also used.

For staining in bulk borax-carmine or paracarmine was employed, the latter being sometimes followed on the slide by picro-indigo-carmine, but without much effect.

I desire to express my thanks to my skilful laboratory assistant, Mr. Charles Biddolph, for the care which he has taken in preparing the sections.

Although many specimens were preserved, only five have

actually been used for the purposes of the present investigation, numbered in my notes 11, 21, 22, 23 and 24 respectively.

It will save repetition if I give particulars concerning these specimens at once, and of the mode of treatment of the material.

No. 11.—A very large specimen from Rum Bay, 80 mm. high by 18 mm. in breadth; brought in about mid-day (April 15th) and examined the same afternoon (about 1 o'clock). When examined a vigorous stream was coming out from the main vent. A section of the living sponge from near the base showed large ova, but no embryos were seen. A section near the vent showed smaller germ-cells. The specimen was cut in half lengthwise, and half fixed in strong Flemming's solution (in the dark) for half an hour, then washed for an hour or more in tap-water and graded through 30 per cent. and 50 per cent. to 70 per cent. alcohol.

No. 21.—A rather small specimen, about 14 mm. high, collected about mid-day on April 18th, and examined the same afternoon. There was an active current issuing from the vent, bringing with it a quantity of fine yellowish-grey sediment, which collected at the bottom of the glass dish. In hand section numerous large rounded cells were seen free in and projecting into the flagellate chambers, apparently actively moving, but probably only owing to the movements of the flagella of the collared cells. Fixed in strong Flemming's solution and preserved in 70 per cent. alcohol.

No. 22.—A moderate-sized specimen, about 25 mm. high by 18 mm. broad; collected about mid-day on April 18th and examined the same aftrnoon. Fixed entire in Flemming and sea-water formol[1] for about a quarter of an hour, then washed in formol and sea-water and preserved in same.

No. 23.—A moderate-sized specimen, collected on April 18th and examined and fixed on the 19th, having been kept in the circulation of the aquarium overnight. Stream coming from osculum when examined. Found no amœbocytes or

[1] Take 10 c.c. commercial formaldehyde in 90 c.c. sea-water and add 20 c.c. strong Flemming's solution.

other cells in water pipetted from gastral cavity. On examination of living section found very numerous rounded amœbocytes with coarse granules, and a few with pseudopodia, in the chambers, some, if not most, attached to walls by short peduncles. Half of specimen fixed in strong Flemming's solution for about an hour, washed and graded up to 70 per cent. alcohol. Another part fixed in absolute alcohol.

No. 24.—A good-sized specimen from Rum Bay, examined and preserved on April 22nd, after having been kept in the aquarium circulation since April 15th. The collared cells were found to be still active and did not show the characteristic signs of suffocation. Bulk of specimen fixed in Flemming and sea-water formol.

(c) The Breeding Season and Life-Cycle.

There is strong, indeed, I think conclusive, reason for believing that Grantia compressa is an annual sponge, growing rapidly during the winter and spring and breaking up and perishing in the autumn, after producing numerous embryos. I have already given particulars as to the sizes of some of the specimens met with at Plymouth in April, 1912, and my colleague, Mr. R. W. H. Row, tells me that about the middle of August, 1913, when he visited Plymouth, they were already breaking up, and it was difficult to obtain a good specimen of any considerable size—indeed, most of them had apparently already disintegrated.

The breeding season at Plymouth would seem to begin in the first half of April; germ-cells are then being produced in enormous numbers, but comparatively few embryos are found. At least that was my experience in 1912.

Previously, in 1911, I had observed mature embryos being shot out of the osculum of a specimen which I examined in the laboratory in the first week of June. I also find plenty of advanced embryos, along with germ-cells in various stages of growth, in a specimen collected for me by Mr. Row about the middle of August, 1913. The germ-cells (ova), however, are nothing like so abundant as in material taken in April.

It seems, therefore, that the breeding season lasts throughout practically the whole of the spring and summer.

According to Mr. Orton, who kindly allows me to make use of information about to be published in the 'Journal of the Marine Biological Association' (1914), there are really two breeding seasons at Plymouth for Grantia compressa. In June embryos are discharged from large specimens (which subsequently disintegrate). These embryos develop into individuals which, while still very small, produce numerous embryos in October. Mr. Orton has also obtained data supporting the view that the same specimen may breed twice during its life-history—once in late autumn and again in the following summer.

(D) The Distribution of the Germ-Cells in the Sponge.

As a result of my observations I think I have been able to establish the fact that Grantia compressa, unlike certain non-calcareous sponges, such as Oscarella lobularis (Schulze, 1877), is hermaphrodite, producing male and female gametes simultaneously. In the case of Sycon raphanus, Poléjaeff, as far back as 1882, came to the same conclusion, but considered that that sponge afforded an example of incomplete sexual separation, some individuals being predominantly male and others predominantly female. The former were extraordinarily rare, but produced an immense quantity of spermatozoa, as well as numerous ova. The latter produced very few, or even (apparently) no spermatazoa, but a large number of eggs.

It is quite possible that the same relations may exist in Grantia compressa, but if so I have never been fortunate enough to find the predominantly male individuals. All that I have examined appear to be predominantly female, the sperm-morulæ occurring only in comparatively small numbers.

Poléjaeff derives both the sperm-morulæ and the ova (in Sycon) from ordinary amœboid wandering cells, and figures

them scattered in the mesoglœa, apparently without any special arrangement.

Görich (1903) also accepts the usual views as to the origin of both male and female germ-cells from amœbocytes, but he states that in Sycon raphanus ova are produced in the lower two thirds and sperm-cells in the upper third of the sponge. He has not, however, followed the spermatogenesis beyond its earliest stages. The only generalisation that I can make about the distribution of the germ-cells in Grantia compressa is that the younger parts of the growing sponge, towards the osculum, only contain immature germ-cells, exactly as might be expected.

It may be admitted that in Grantia compressa also the germ-cells, both male and female, can be traced back to amœboid wandering cells, but, according to my own observations, these amœbocytes can, in their turn, be traced back to collared cells of the gastral epithelium lining the flagellate chambers. Presumably any collared cell may become directly transformed into a primary oögonium or spermatogonium, losing its collar and flagellum and becoming amœboid. The evidence for this statement will be presented in the next section.

Having become amœboid, the young germ-cells are free to wander about. The primary oögonia first migrate into the mesoglœa from the gastral epithelium, and later on, when fully grown, they migrate back into the chambers, where they undergo repeated division and give rise to small oöcytes. The young oöcytes remain, feeding and growing, for some time in the chambers; then they migrate once more into the mesoglœa, where they undergo enormous growth, followed by maturation and fertilisation.

The migrations of the spermatogonia appear to be of a less extensive character. I have reason to believe that they may migrate into the mesoglœa and there become provided with their cover-cells or spermatocysts, but they appear to spend most of their existence in, or attached to, the walls of the flagellate chambers.

It is easy to observe, in hand-cut sections of living specimens in the early part of the breeding season, that the flagellate chambers contain large numbers of amœbocytes hanging, as it were, from their walls. These are, for the most part, germ-cells of both sexes in various stages of growth, although other amœbocytes may also occur in the chambers.

There is thus no localisation of the germ-cells in Grantia compressa, nothing that can be spoken of as gonads, neither ovaries nor testes. Just before undergoing maturation, however, the relatively enormous ova withdraw their pseudopodia and round off, each one taking up a definite position behind the gastral epithelium of an adjacent chamber, and causing the layer of collared cells to bulge out into the chamber. Here fertilisation and the earlier stages of development take place, the embryo becoming surrounded by an endothelial capsule, derived from the mesoglœa, during the latter process. Finally the ciliated amphiblastula breaks through the layer of collared cells into the chamber cavity, and is discharged into the sea through the central gastral cavity and vent.

The sperm-morulæ are also discharged into the flagellate chambers, and doubtless find their way out through the vent. I have found them, not only in the chambers, but also adhering to the outer surface of the sponge and in the inhalant canals, though, except in the chambers themselves, only in very small numbers. I have also found some evidence of their breaking up into spermatozoa in an inhalant canal.

There can be little doubt that fertilisation is effected by spermatozoa which enter the sponge (perhaps as sperm-morulæ) through the dermal pores with the inflowing stream of water, but whether these spermatozoa are derived from the same sponge as the eggs which they fertilise, or from another individual, would seem to be a matter of pure chance.

In a paper on the "Anatomy of Grantia labyrinthica, etc.," published in 1891, I expressed the opinion that the ova migrated through the walls of the inhalant canals and were

fertilised while suspended in the inflowing stream of water, subsequently migrating back to undergo their development in the mesoglœa. I certainly did observe amœbocytes suspended in this position, but I now realise that they were far too small to be mature ova, and must unreservedly withdraw my interpretation of the observation. It is evident that maturation and fertilisation (in Grantia compressa) both take place after the ovum has taken up its definitive position in the mesoglœa behind the gastral epithelium. Possibly the spermatozoon has to penetrate a thin layer of dermal epithelium and mesoglœa in order to reach the ovum, or perhaps the presence of the enormous ovum causes some rupture in the wall of an adjacent inhalant canal. It is impossible to say exactly what takes place.

One more point may be mentioned in this section, and that is the tendency of particular stages of gametogenesis, or at any rate of oögenesis, to occur in large numbers in certain specimens, or in certain parts of the sponge, while more or less completely absent from others. Instances of this phenomenon will be given in the following pages; it seems to indicate that the oögonia are produced in successive crops which go through their developmental stages synchronously.

(E) The Relations between the Different Tissue Elements.

There can be no doubt that the tissues of sponges are far less definite and less permanent than those of typical Metazoa. Without going back to the old view that the sponge is nothing more than a colony of Protozoa, which seems to be negatived by the degree of histological differentiation that they exhibit and by the facts of sponge embryology, we may safely say that the individual cells of which the sponge is composed often exhibit a remarkable power of changing their relative positions and also a high degree of polymorphism. Thus it will be remembered that Minchin (1898) has shown that the cells (scleroblasts) which secrete the triradiate spicule-systems in calcareous sponges migrate into the mesoglœa from the

dermal epithelium ("ectoderm"), and that the porocytes in Leucosolenia, when the sponge contracts, migrate through the gastral epithelium and fill up the central cavity (Minchin, 1900). It is also well known that the epithelial cells of the so-called ectoderm are highly contractile and capable of great change of shape, and Maas has shown (1900) that in the developing Sycon the epithelial cells lining the central gastral cavity are derived by immigration from the dermal epithelium on the outer surface of the sponge.

In the case of Grantia compressa it is hardly possible to speak of permanent tissues at all. According to my observations any of the constituent cells of the sponge may become amœboid and wander off to some new situation. This may very easily be demonstrated for the collared cells by examining teased preparations of the living sponge in sea-water, when the collared cells can be seen putting out long, hyaline, finger-shaped pseudopodia in an extremely characteristic manner.[1] The fact that the collar may still be present along with the pseudopodia, as shown in fig. 1, affords unmistakable proof of the origin of these amœboid cells in teased preparations. In stained sections we sometimes see something of the same kind, and fig. 2 represents a collared cell sinking into the mesoglœa from between its fellows of the gastral epithelium. In this case pseudopodia and flagellum are seen to be present simultaneously but the collar is not visible. In fig. 3 an amœbocyte, probably derived from a collared cell, but apparently without collar, flagellum and pseudopodia, is seen lying in the mesoglœa behind the gastral epithelium.

Appearances such as are represented in figs. 4, 6 and 7 also indicate very clearly that the cells of the so-called ectoderm are not only contractile, but may become converted into amœbocytes and wander off into the mesoglœa. In fig. 4 is shown one of the epithelial cells lining the central gastral cavity in the contracted or "flask-shaped" condition (*a*), and in the subjacent mesoglœa a typical amœbocyte (*b*). Fig. 6 shows how such amœbocytes may be directly derived from

[1] Compare Carter (1875, p. 22) for a similar observation.

epithelial cells which have migrated inwards, the identity of the two being clearly indicated by the presence of the numerous darkly stained granules that characterised the epithelial cells of the central gastral cavity, at any rate in this specimen. Fig. 7 shows a similar relation between the much less granular epithelial cells and amœbocytes around an inhalant canal.

The mesoglœa of Grantia compressa contains, of course, a large number of amœbocytes, and there is no need to suppose that all of them are merely amœboid phases of either collared or pavement epithelial cells (compare figs. 58–61). Sometimes small amœbocytes may appear to form connective-tissue networks of stellate cells, but I doubt very much if they really do so, at any rate more than temporarily, and even in this condition they bear such a close resemblance to the epithelial cells lining the inhalant canals that I entirely fail to see how they can be distinguished cytologically.

Under these circumstances it is, of course, quite impossible to say what cells of the adult sponge are derived from each of the cell-groups recognisable in the larva. It also seems quite inadequate to say that the germ-cells are derived from wandering cells in the mesoglœa.

The one constant and characteristic feature about sponge histology is, of course, the collared cell, and that is only constant in the sense that its typical form is that which possesses collar and flagellum. The sponge is, after all, not very much more highly advanced in organisation than a colony of choano-flagellate Protozoa. In such a colony we should certainly, I think, expect the germ-cells to be derived from collared cells, either directly or indirectly through an amœboid phase, and in a later section I hope to be able to show that this is how they actually originate in sponges.

There is one feature about the collared-cells which appears to have attracted but little attention from sponge histologists but which deserves notice in this connection. I refer to the accumulation in them of what appear to be granules of reserve food-material. I have observed these as a very constant

feature in sections stained in a variety of ways, as polygonal bodies scattered more or less abundantly in the cytoplasm (figs. 2, 3, 8, etc.). They vary much in size and in the intensity with which they stain. In sections of material (spec. 23) fixed in absolute alcohol and stained with borax-carmine followed by picro-indigo carmine they are distinctly recognisable, and stain a pale, greyish colour. In sections of Flemming material without further staining they can easily be detected, though only very lightly stained.

They are quite distinct in Flemming material stained with iron-brazilin, but, I think, more so after counter-staining with picro-indigo carmine, when they appear of a dark grey colour (spec. 11). They are hardly affected by nuclear stains and are evidently not chromidial in nature. The depth to which they stain exhibits a curious variation in some cases, as will be seen by reference to fig. 8, from a section of material (spec. 21) fixed in strong Flemming and stained with paracarmine and picro-indigo carmine, where variation in this respect is visible even in one and the same cell. They are usually most abundant in the lower part of the cell (figs. 3, 8).

These observations on their staining reactions appear to me to be quite in harmony with my view that the bodies in question are "reserve granules." The question next arises, Are such granules characteristic of the collared cells, or do they occur also in the other tissue-elements? We have already had occasion to notice the presence of numerous granules in the epithelial cells lining the central gastral cavity and in the amœbocytes derived from these (fig. 6). These granules, it will be observed, are of a different character from those now under consideration, being smaller and more highly refractive. My observations lead me to believe, however, that granules similar to those in the collared cells may also occur in the epithelial cells both of the gastral surface and of the inhalant canal system, though perhaps less abundantly. They certainly occur in many of the amœbocytes, and I had at first hoped that their presence might have served as a means of distinguishing those amœbocytes that originate from collared

cells from those that do not. I fear, however, that this hope must be largely abandoned, though the granules in question may perhaps serve as supplementary evidence of origin in certain cases.

Another point to be noticed in connection with the collared cells concerns the condition of the nucleus. Usually in my sections this appears to be darkly and almost uniformly stained, but frequently it exhibits a distinctly reticulate character, with small, scattered, chromatin granules at the nodes of the reticulum, while intermediate conditions occur between the two extremes. As all conditions may occur close together in the same preparation it is difficult to account for the differences, but such variations have to be borne in mind in considering the origin of the germ-cells as indicated by nuclear characters.

Miss Muriel Robertson and Prof. Minchin (1910) have given an account of the division of the collared cells in Clathrina coriacea, and Miss Robertson (1911) has dealt with the corresponding phenomena in Grantia compressa and Sycon sp. Though figuring the collared cells in detail, neither of these authors refer to the "reserve granules," which I find to be such a constant feature in Grantia. This is perhaps due in part to the fact that their preparations were stained especially with a view to demonstrating the phenomena of mitosis. They figure much the same variations in the appearance of the nuclei of the collared cells as I have seen, but I cannot agree with their views as to the general occurrence of a single, relatively large karyosome (nucleolus). I have myself only occasionally seen such a body in these nuclei and do not attribute any special importance to it.

Alike in Clathrina, Sycon and Grantia the division of the collared cells was found by Robertson and Minchin to take place longitudinally and to be accompanied by a typical mitosis. In Clathrina the number of chromosomes was found to be "about sixteen," but in Grantia and Sycon the chromosomes are said to be "not very distinct," and the number is not given. Miss Robertson's figures, however, especially

fig. 12 on Pl. 19, suggest eight or ten as the number rather than sixteen. I have myself hardly ever observed mitosis in the collared cells, but my fig. 9 represents a probable case, and it will be seen that so far as my very limited observations go they agree with those of Miss Robertson.

I do not, of course, profess to give a complete account of the collared cells in this place; much more might be said about them, but the only thing needful here is to emphasise those points which have a direct bearing upon the problem of the origin and maturation of the germ-cells.

(f) Oögenesis.

(a) Historical.

By far the most complete account that has yet been given of the oögenesis in any sponge is contained in Dr. Max Jörgensen's memoir (1910). As this account refers to a type (Sycon) closely related to Grantia compressa, I propose to give a brief review of Jörgensen's results before passing on to describe my own.

As already noticed, this author derives the primary oögonia from so-called mesoderm cells, either resting stellate connective-tissue cells, or amœbocytes, between which he considers that no essential difference exists. The mesoderm cells multiply mitotically and become converted into oögonia of the first order. These increase in size and presently wander into the flagellate chambers, where they undergo mitosis and divide into oögonia of the second order. The latter divide again into oöcytes. The two oögonial divisions are said to be "atypical," but quite similar to one another. During the mitosis eight chromosomes make their appearance "in Form von Tetraden"; these are believed to be formed by fusion of several segments of a segmented spireme. Each "tetrad" is figured as dividing into two tetradiform daughter-chromosomes on the spindle.

Although the division of the oögonia frequently occurs in

the flagellate chamber, the author evidently regards this as a more or less accidental circumstance, which takes place only if the öogonia have not got room enough to divide in the mesoglœa. I think myself that the migration of the oögonia into the chambers before dividing must have a deeper significance than this.

After their divisions are over the daughter-cells of the oögonia wander back into the mesoglœa as young oöcytes. Here they undergo a short resting stage, and then they pass through the prophases of what must apparently be regarded as the mitosis belonging to the first maturation division. A long spireme is formed which arranges itself in a characteristic "bouquet" form, and even shows a contraction phase similar to synapsis, though this may be due to the action of reagents; at the same time "chromidia" appear in the cytoplasm, probably ejected from the nucleus. A large nucleolus is likewise present, but this is also a characteristic feature of the oögonia. Some of these young oöcytes now degenerate, possibly furnishing nutrient cells for the older oöcytes. In others the spireme breaks up again into chromatin granules, and the oöcyte continues its growth.

In the later stages the oöcyte increases enormously in size and puts out long, branching pseudopodia. When the growth of the cytoplasm has reached its completion the nucleus enters upon the so-called "critical stage," in which the nucleolus has completely disappeared and the nucleus is almost entirely devoid of chromatin. Apparently the chromatin has migrated through the nuclear membrane into the surrounding cytoplasm, where it is represented by "chromidia." Chromatin-granules and nucleolus now reappear in the nucleus, which becomes very large and vesicular. Then a second diminution in the amount of chromatin takes place, this time apparently effected by solution or absorption within the nucleus itself and not by extrusion into the cytoplasm. The nucleus, with its diminished quantity of chromatin arranged once more in the form of tetrads, approaches the surface of the oöcyte, which has in the meantime rounded

itself off. The nuclear membrane now disappears, and the eight tetradiform chromosomes, now much diminished in size, arrange themselves on the equator of the first maturation spindle. Each chromosome divides into two daughter tetradiform chromosomes and the mitosis is completed in the ordinary way. Then the first polar body, containing eight of the daughter-chromosomes ("tetrads"), is twisted off from the surface of the oöcyte in a very characteristic manner. A second maturation division now takes place and eight chromatin elements finally remain in the fertilised egg, eight having passed out into the second polar body, but the formation of the second polar body was not fully observed.

It is difficult to understand completely the author's views as to the manner in which the reduction of the chromosomes is carried out, and, indeed, although he discusses the problem at some length, he does not profess to have come to any certain conclusions: "Leider ist mein Objekt zu klein und ungünstig, um diese wichtige Frage sicher zu entscheiden." At any rate he evidently considers that the somatic number of chromosomes is sixteen and the reduced number eight, for he finds sixteen in the segmentation nuclei of the embryo. The most curious thing appears to be that the number is already reduced to eight in the oögonial mitoses, but it is suggested that this may be a pseudo-reduction. Possibly it is connected with the tetrad formation which is supposed to take place at this stage.

The author describes an interesting process of nutrition of the growing oöcyte by ingestion of nutrient cells. I have observed a somewhat similar phenomenon myself in the case of Grantia and shall discuss it at length later on.

As regards the fertilisation of the ovum and the subsequent segmentation stages the most interesting feature appears to be the splitting up of the pronuclei and segmentation nuclei into karyomeres, a phenomenon which I have also observed in Grantia.

(b) Origin and Growth of the Primary Oögonia.

Before proceeding to describe the origin and growth of the primary oögonia as observed in Grantia compressa it may be well to point out the great difficulties that arise with regard to the problem of seriation in the earlier stages of oögenesis. These difficulties are accentuated by the absence of definite localisation of the germ-cells and the consequent mingling of different stages in the mesoglœa or in the chambers, so that it is often impossible to be certain even to which generation a particular cell belongs. We can only fit the different observed stages together in what seems to be the most probable order on the sometimes scanty evidence available.

In the later stages of growth of the oöcyte there is less difficulty, because the size of the cell not only shows that it is an oöcyte, but indicates at the same time its place in the series.

Inasmuch as amœboid cells of various sizes frequently immigrate into the chambers and come to lie between the collared cells, the mere fact of the occurrence of a young germ-cell in such a position affords no conclusive evidence that it has been derived from a collared cell. The only way in which such an origin can be demonstrated, as it seems to me, is by finding cells which exhibit the characters of young germ-cells while still retaining the collar or flagellum, or both, of the collared cells. It must be admitted that it is not often that such intermediate forms are met with, and it is obvious that they can only be hoped for in very carefully prepared sections. I have, however, seen a few such cases, which seem to me to place my conclusions almost beyond doubt. Figs. 10, 11, and 12 are all taken from sections of specimen 21, stained with paracarmine and picro-indigo carmine. In Fig. 10 two collared cells are represented side by side. In both the collar and flagellum are clearly visible, and in one the body of the cell is already considerably enlarged, apparently by the accumulation of reserve material. This enlarged collared cell I take to be a primordial germ-cell, but

whether it would have turned into an oögonium or a spermatogonium cannot be decided. The appearance of a vacuole around an unusually large granule of reserve material, however, suggests to my mind the latter.[1]

Figs. 11 and 12 represents a later stage, in which the body of the cell is greatly distended with reserve granules similar to those which occur in the ordinary collared cells, and the remains of collar and flagellum are, unless I am mistaken in my interpretation of the appearances, still visible. The nucleus has retreated to about the middle of the cell and a distinct nucleolus or karyosome has appeared. The whole cell projects conspicuously beyond its neighbours into the cavity of the flagellate chamber, and there is a clear indication of pseudopodium formation represented in fig. 12, the pseudopodium being formed by a drawing out of the proximal end of the cell between the adjacent collared cells. (An apparent pseudopodium shown in fig. 11 may belong to another cell not seen in the section.) In fig. 11 it will be seen that the young oögonium lies actually next to the exhalant aperture of the chamber, and the one represented in fig. 12 also lies close to an exhalant aperture, but I have not sufficient evidence to show whether or not there in any constancy in this position.

The primary oögonia appear to leave the layer of collared cells very soon after their origin and migrate into the mesoglœa (fig. 13). The collar and flagellum completely disappear and pseudopodia are put out (fig. 14). The nucleus at first appears uniformly stained except for the large nucleolus, but presently small granules of chromatin appear scattered between the nucleolus and the nuclear membrane, though the nucleolus appears to be surrounded by a narrow ring free from granules (fig. 15). The reserve granules are still abundant and easily recognisable in the cytoplasm.

The primary oögonium now appears to round itself off more or less completely before entering upon mitosis, as shown in

[1] Compare the account of the origin of the primary spermatogonia, later on.

fig. 16. In specimen 22 the mesoglœa between the chambers is crowded with oögonia in this condition, and their numbers suggest that it may be a resting state. It will be seen that the cytoplasm is only slightly granular, but may still contain reserve material in the form of polygonal granules. The nucleus is faintly reticulate, and there is a very large and conspicuous nucleolus and a thin nuclear membrane.

(c) Multiplication of the Oögonia.

Having reached the stage represented in fig. 16 the oögonia begin to prepare for division, which is effected by mitosis. During the progress of this mitosis they migrate through the layer of collared cells into the flagellate chambers (fig. 21), where the cell-division actually takes place. Although the earlier stages of the mitosis are found in the mesoglœa (figs. 17, 18), I have never seen the actual division taking place except in the chambers themselves, and cannot therefore agree with Jörgensen that the oögonia only migrate into the chambers when they are short of room in the mesoglœa. This view is not in harmony with the fact that the fertilised eggs, which are many times larger than the oögonia, manage to find room for their development in the mesoglœa by pushing out the gastral epithelium without rupturing it. I therefore think that there must be some special reason for the migration of the oögonia into the chambers. I would suggest that it may enable the young oöcytes to find abundant nutriment in the chambers in the first instance, and subsequently to distribute themselves more readily throughout the sponge by creeping along inside the walls of the chambers and re-entering the mesoglœa at various points.

Jörgensen is of opinion that there are two generations of oögonia in Sycon, and the large number of oöcytes present seems to indicate that there must be at least two oögonial divisions in Grantia. Further evidence of this is to be found in the small size of the youngest oöcytes as compared with the daughter-cells formed by division of the primary oögonia (compare figs. 29 and 26).

Before proceeding to describe the mitosis of the primary oögonium it is necessary to say a few words with regard to the character of the chromatin substance in the nucleus. The growing oögonium contains, as we have seen, a large spherical nucleolus or karyosome (fig. 14, etc.), which stains very darkly, and in its later stages at any rate minute granules of chromatin may also appear in the nucleoplasm (fig. 15). During the prophases of mitosis all the darkly staining granules of chromatin disappear from the nucleus, while the nucleolus may be cast forth from the oögonium altogether (figs. 17, 18). There remains behind a quantity of more lightly staining chromatin which forms the spireme, and, subsequently, the chromosomes. It is, I think, impossible to avoid the conclusion that there are here two totally different kinds of chromatin, for which we may accept the terms "trophochromatin" and "idiochromatin" respectively, the former being concerned in the metabolism and growth of the cell and the latter in the processes of reproduction. The trophochromatin is represented chiefly by the nucleolus. Small granules of chromatin are possibly cast out into the cytoplasm as chromidia, for there is some evidence that chromidia may be found at this stage, but they are nothing like so conspicuous as they are in the oöcyte. In the latter the chromidia are, of course, supposed to be concerned in yolk-formation, which has hardly commenced in the oögonium. The large nucleolus appears to be bodily cast forth from the oögonium during mitosis (fig. 18). It can, therefore, hardly be supposed to be directly concerned in yolk-formation at this stage. It may possibly be of a different nature from the chromidia, and represent a mass of waste products accumulated in the nucleus during the growth and metabolism of the oögonium. In the young oöcyte, however, as we shall see shortly, the nucleolus definitely gives rise to chromidia ("yolk nucleus"), while in the maturing oöcyte it appears to undergo degeneration and absorption in the cytoplasm.

Jörgensen gives an interesting discussion on the nature and behaviour of the chromatin in the oöcytes of Sycon, to which

I must refer the reader, but he appears to have paid very little attention to this question so far as the oögonia are concerned, and does not seem to have observed the bodily ejection of the nucleolus.

We may also say a few words here with regard to the formation of the chromosomes. Jörgensen figures both spireme and chromosomes as being stained perfectly black—as black, in fact, as the chromidia. His material, like mine, was fixed in Flemming's solution, and he used iron-hæmatoxylin for staining (controlled by borax carmine and saffranin preparations). My own preparations were for the most part stained with iron-brazilin, but I have also used iron-hæmatoxylin. There is, of course, a good deal of variation in the results obtained by either of these methods, but my experience is that, as a rule, the spireme thread and chromosomes stain comparatively lightly as compared with the nucleolus and chromidia. I have never seen the chromosomes so sharply defined as Jörgensen figures them, and I have seen nothing of the so-called tetrad formation which he describes during the oögonial mitoses and in the maturing oöcyte. The chromosomes have always appeared to me much more like those figured by Prof. Minchin and Miss Robertson for the dividing collared cells—small subspherical or irregular bodies, so crowded together and ill-defined that it is impossible to count them accurately. The number characteristic of the oögonia appears to be about eight, as will be seen by reference to figs. 19–23, and this is possibly the somatic number (compare fig. 9).

After these preliminary observations the actual division of the primary oögonia may be described very briefly. The prophases of the mitosis occur while the oögonium is still lying in the mesoglœa outside the chamber which it is about to enter, and while it is in a more or less amœboid condition (figs. 17, 18). Whether or not there is any casting out of chromidia into the cytoplasm I am not certain, but as a few small, densely staining bodies resembling chromidia sometimes appear in the cytoplasm in later stages of this mitosis, it

seems not unlikely that such may occasionally be the case. Apart from the nucleolus, however, there is very little chromatin in the nucleus to be cast out.

The large vesicular nucleus approaches the surface of the oögonium until it is bounded on the outside by only a very thin layer of cytoplasm. In the meantime a spireme thread makes its appearance, and the nucleolus also approaches the surface (fig. 17). The nuclear membrane disappears, and the nuclear sap merges into the cytoplasm. A very curious phenomenon now takes place, the nucleolus being expelled, not only from the nucleus, but from the oögonium. Fig. 18 shows it in the process of extrusion, surrounded by a drop of nuclear sap. The pear-shaped form of the nucleolus at the moment of extrusion suggests that it must be a very soft, perhaps a semi-fluid, body in life. I have only seen this phenomenon exhibited very rarely in what can be considered as at all a conclusive manner. I have frequently seen the nucleolus apparently cast out of the oöcyte, but minute inspection shows that this is (? always) an artificial result brought about in the act of cutting the sections. During the process of fixation, etc., the nucleolus appears to become very hard, and the knife then tears it bodily out of the oöcyte. In the case represented in fig. 18, however, I think there can be no question of the normality of the process of extrusion; indeed, that the nucleolus must be extruded at this stage seems to be indicated by its complete absence in later stages of the mitosis.

It is extremely difficult to determine whether a particular spireme stage under observation belongs to an oögonial or to an oöcyte mitosis. I am inclined to think, however, that in the former case there are few or no chromidia, while in the latter the chromidia are fairly strongly developed (cf. figs. 44, 45). I must admit again, however, that the sorting out of these stages is to a large extent arbitrary.

The spireme thread now breaks up into chromosomes, a typical spindle is formed with a minute centrosome at each pole, and the chromosomes arrange themselves in the usual

"equatorial plate" (figs. 19, 20). The chromosomes now presumably divide, though I can hardly claim to have seen the actual division (cf., however, fig. 21), and the two groups of daughter-chromosomes migrate towards the two centrosomes (figs. 22, 23). The spindle disappears, and we are left with two closely aggregated groups of chromosomes as the foundations of the two daughter-nuclei (fig. 24). Constriction of the cytoplasm between these two daughter-nuclei now follows (fig. 25), and finally the entire oögonium becomes divided into two daughter-cells (fig. 26).

Apparently not until the prophases have been passed through does the oögonium migrate through the layer of collared cells into the adjacent flagellate chamber, as shown in fig. 21. Here it rounds itself off, usually into an oval form (figs. 19, 20), before completing the mitosis. At this stage the cytoplasm exhibits a fairly uniformly and densely granular character, shown especially well in fig. 22. A few small densely staining granules, resembling chromidia, are sometimes visible in it (fig. 19), and may even persist in the daughter-oögonia after completion of the division (fig. 26).

It seems almost certain that a second oögonial division takes place in the flagellate chambers very shortly after the first one. Fig 27 represents a stage which I interpret as an oögonium of the second generation with reconstituted nucleus, and fig. 28 represents what I take to be such a secondary oögonium in mitosis. Without the intervention of such a stage it would be difficult to explain the origin of the next series of stages (figs. 29–39), which occur very abundantly in the chambers, and which I interpret as young oöcytes. With regard to this period of the oögenesis my conclusions differ widely from those of Jörgensen, who makes his oögonia of the second order larger than those of the first order, and his young oöcytes larger still, which would be very difficult to understand in view of the repeated division, and the apparent absence, so far as his account goes, of any process of nutrition.

(d) Growth and Feeding of the Young Oöcytes in the Flagellate Chambers.

The growth of the oöcytes may be divided into two very distinct periods, during the first of which they are found in the flagellate chambers, while during the second they lie in the mesoglœa between the chambers.

As already indicated, it is by no means an easy matter to sort out all the very numerous amœboid cells that occur in the flagellate chambers, some representing stages in oögenesis and others stages in spermatogenesis, into their proper categories. Amongst them, however, may be distinguished a type which occurs very abundantly and exhibits certain peculiarities by which it is more or less readily recognised. The cells in question are small and distinctly amœboid, of irregular form, and usually attached to the wall of the chamber by pseudopodia. They have a rather small nucleus, with a relatively large nucleolus surrounded by a narrow clear space, and then by a broad ring of minute granules extending to the nuclear membrane. The cytoplasm usually exhibits more or less numerous inclusions which may be surrounded by vacuoles. Some of these inclusions, which I interpret as chromidia or yolk-nuclei, stain nearly black, others may stain much more lightly, and look like food-particles undergoing digestion. A typical series of these curious cells is shown in figs. 29–39. I interpret them as young oöcytes engaged in feeding operations.

Figs. 29 and 30 show what appears to be the youngest stage of this series, a stage which may be derived from the mitotic division of a secondary oögonium such as is represented in fig. 27 or 28. The cytoplasm at this stage is seen to be uniformly and rather coarsely granular and contains no chromidia or other inclusions of any kind. The structure of the nucleus even at this early period, with its large nucleolus and broad zone of chromatin granules distinctly separated from it, appears to me to be essentially that of an immature female gamete. Presently the characteristic inclusions make

their appearance in the cytoplasm, which otherwise may come to exhibit a more homogeneous appearance (figs. 31–39). Some of these inclusions are comparatively lightly staining bodies enclosed in vacuoles (figs. 31, 36, 37), and in one case (fig. 39) a nucleated cell was distinctly recognised amongst other bodies. I therefore believe that the lighter coloured inclusions are food-particles undergoing digestion, captured by the young oöcytes from the stream of water that flows through the chambers.

The nature of the intensely black stained bodies that appear in the cytoplasm is easily interpreted. These resemble the nucleolus in appearance, but may be much larger (figs. 33–35). They may or may not be surrounded by distinct vacuoles. On the other hand, they may take the form of small granules or groups of granules (figs. 35, 36). Fig. 38 gives the clue to the manner in which they are formed, for here the nucleolus is seen actually discharging part of its own substance into the cytoplasm through the nuclear membrane. I think there can be no doubt that in these cells very active metabolism, accompanied by the formation of chromidia, or "yolk-nuclei," is going on, and that the bodies in question are of this nature, and are concerned in the elaboration of yolk-granules in the cytoplasm.

Apparently feeding now ceases and the remains of food-particles disappear from the cytoplasm. The oöcyte next migrates through the chamber wall into the mesoglœa (fig. 40).

(e) Growth and Feeding of the Oöcytes in the Mesoglœa.

The formation of chromidia, or "yolk-nuclei," by extrusion of matter from the nucleolus, which was already commenced within the chambers (fig. 38), may now be continued very freely. Specimen 23 contains an immense number of oöcytes, lying in the mesoglœa between the chambers, in which this chromidium-formation is going on (figs. 41, 42, 43), and often giving rise to very fantastic appearances. The nucleolus is

evidently in a liquid or semi-liquid state and appears to be squeezed out into the cytoplasm in threads or drops, just as an artist's colours may be squeezed out of their tubes. During this process the nucleus itself may become distinctly pear-shaped (fig. 43). The drops squeezed out into the cytoplasm are at first enclosed in distinct vacuoles, apparently derived from the nuclear sap. In these vacuoles they disintegrate into granules (fig. 43), which probably became scattered through the cytoplasm. The nuclear membrane may become very indistinct during the process, and there are indications that the nucleus is passing into the spireme stage to be described next. It is doubtful whether the nucleolus is ever completely eliminated from the nucleus at this stage. It seems to me more probable that some of it always remains behind as the foundation of the huge nucleolus which forms such a conspicuous feature in later stages of the oöcyte. The irregularity in shape of the oöcyte during this process of chromidium formation indicates that it is still amœboid, and it seems possible that the extrusion of the chromidia may be due to strong contraction of the cytoplasm, though it must be admitted that the mechanism of the process is very obscure.

The whole of the series of stages representing the feeding and growth of the young oöcytes in the chambers and the remarkable process of chromidium-formation just described appears to have been unobserved by Jörgensen. It is true that that observer worked upon Sycon, but it is unlikely that two such closely related types as Sycon and Grantia should differ in this respect, especially when they agree so closely as regards other features of the oögenesis. Jörgensen figures the young oöcyte, supposed to be directly derived from the last oögonial division, as being very similar to the stage represented in my fig. 47a (compare his fig. 32). This stage may very easily be derived, however, through the intermediate stages represented in figs. 41–43, from the last of the feeding stages observed in the chambers (fig. 39).

It also seems very probable that shortly after leaving the flagellate chambers and undergoing the process of chromidium-

formation just described, the young oöcyte exhibits the prophases of a mitosis which really belongs to the first maturation division. Jörgensen puts this incomplete mitosis, represented by a well-marked spireme stage, immediately after the stage which, in Sycon, probably corresponds to my fig. 47a. I prefer to place it immediately before this stage, where it seems to me to fit in better. Figs. 44 and 45 show two of the prophases in question. The former is evidently a leptotene phase and the latter a pachytene. The latter always shows the characteristic contraction of the spireme described by Jörgensen, which may possibly represent a synapsis or be due simply to the action of reagents. Both these figures show well-developed chromidia in the cytoplasm, which, as I have already said, inclines me to include them in the oöcyte series rather than in the oögonial series, though I do not consider that the evidence is by any means conclusive. It is obvious from fig. 45 that the oöcyte may still be highly amœboid during this phase, exhibiting a very irregular outline.

Jörgensen considers that some of the oöcytes at this stage undergo degeneration and may have something to do with forming the nutrient cells for the older oöcytes, but I have obtained no good evidence of such degeneration and my observations on the feeding of the older oöcytes do not support this view. The spireme thread now disappears (fig. 46) and the oöcyte rounds itself off, takes up a definite position in the mesoglœa behind the layer of collared cells (fig. 47), and enters upon its main period of growth.

Fig. 47a represents a condition of the oöcyte which is very commonly met with. Jörgensen figures a similar condition in Sycon (fig. 32), and speaks of it as a resting condition, but, as I have already pointed out, he regards it as the direct product of the division of an oögonium of the second generation. I am not quite sure, however, that Jörgensen's fig. 32 really represents the same stage as my fig. 47a, for he does not show, or if so only very faintly, the chromidia in the cytoplasm, which appear to me to be very characteristic of this stage. It is by the abundance of these chromidia,

indeed, that this stage is chiefly distinguishable from the primary oögonium just before mitosis, as represented in fig. 16, taken in conjunction with the fact that the latter occurs especially in association with the oögonial mitoses going on in the chambers, while the former occurs especially associated with the later stages of oöcyte growth.

In Grantia the young oöcyte, passing out of this "resting condition," simply flattens out somewhat on one side (fig. 47b), and puts out long branching pseudopodia by means of which it attaches itself to the mesoglœal surface of the layer of collared cells, as shown in fig. 48. Whether these pseudopodia are merely "anchoring" pseudopodia, or whether they also serve to extract nutriment for the growing oöcyte from the collared cells with which they are in contact, must for the present remain an open question. The appearances represented in fig. 48, however, suggest to my mind the latter. We are reminded of the manner in which the superficial cells of the embryo of Stelospongus attach themselves to, and evidently draw nutriment from, the large capsule-cells by which they are surronnded, as described by me many years ago (Dendy, 1888). It will be seen that the collared cells contain abundant "reserve-granules," and similar granules appear in the oöcytes, while the chromidia have entirely disappeared from the cytoplasm. The nucleus is distinctly reticulate, with numerous darkly stained chromatin grannles and a large nucleolus, the latter surrounded by a clear space (possibly due to shrinkage ?).

The oöcyte continues to increase in size and the nucleus grows more rapidly than the cytoplasm. Presently we reach a stage which is very characteristic and very frequently met with, and which I propose to call the "contraction stage of the oöcyte." This is represented in fig. 49. It will be seen that the entire cell has rounded itself off again and the pseudopodia have contracted into blunt knobs. The nucleus is very large and pretty uniformly granular, all or nearly all the darkly staining chromatin having evidently been expelled into the cytoplasm in the form of chromidia (yolk-nucleus),

which at this period are apparently not derived, at any rate directly, from the nucleolus. The latter is very large, spherical, and fairly darkly staining. This stage, again, does not appear to have been observed by Jörgensen in the case of Sycon.

It is at about this period that the feeding of the oöcyte by means of nurse-cells begins, a process which continues right on until the oöcyte has reached its maximum size or nearly so, and which will be dealt with separately in a later section (see, in the meantime, figs. 50, 51, 52).

During this process the oöcyte increases enormously in size, and long, root-like pseudopodia are put out again (fig. 54) in a plane parallel to the layer of collared cells against which the oöcyte lies. The nucleus assumes the form of an enormous, thin-walled vesicle, containing a reticulum of lightly staining, flocculent material, which looks very much like a precipitate or coagulation, and includes small, darkly staining chromatin granules scattered through it. Most of the darkly staining chromatin, however, appears to be expelled into the cytoplasm in the form of chromidia, which may be extremely numerous (fig. 53). The nucleolus is very large, and frequently exhibits a differentiation into more and less darkly staining spheres, which may or may not be concentric (figs. 52, 53, 54). Occasionally the nucleolus appears to be cast out into the cytoplasm, but, as already stated, I have come to the conclusion that this is an artificial result, due to the action of the knife in cutting. Sometimes the cytoplasm around the nucleus exhibits a faint radial arrangement of its granules, as shown in figs. 52 and 54. This is a feature upon which Jörgensen has laid some stress in the case of Sycon.

(f) Maturation of the Oöcytes.

The oöcyte now withdraws all its pseudopodia and rounds itself off into a compact ellipsoid body about 0·045 mm. in maximum diameter, preparatory to maturation. The huge vesicular nucleus disappears and its contents are apparently

diffused throughout the cytoplasm, which is dense, and, with the exception of certain inclusions to be mentioned immediately, uniformly and rather coarsely granular. At no stage have I seen any vitelline membrane. The cytoplasm contains a large number of very small chromidia, mostly aggregated in irregular groups or clouds (fig. 55), which are probably, in part at any rate, derived directly from the small chromatin granules that remained in the nucleus at the close of its career. The immense nucleolus in two cases (out of the very few met with) was clearly discernible in the cytoplasm, where it appeared to be undergoing absorption (fig. 55, *no.*). In one case a group of small bodies that might be chromosomes (fig. 55, *chr. ?*) was observed in the cytoplasm at some distance from the nucleolus; but the nature of these bodies is really uncertain, as also is the nature of a protrusion of the surface of the oöcyte opposite to the supposed chromosome-group. It is possible that we have here the beginning of the formation of the first polar body, but I think that that is extremely doubtful.

At a slightly later stage, however, the chromosomes appear unmistakably in connection with the first maturation spindle, which is represented in fig. 56. It is obvious that this agrees closely with the first maturation spindle as described by Jörgensen (cf. his fig. 59), but unfortunately I have only been able to find one really good maturation spindle in my preparations, and I am therefore unable to give any details with regard to the process. I am not even sure of the number of chromosomes, but there appear to be eight or ten in each daughter-group represented in fig. 56. Jörgensen makes the number eight in each group, but represents each chromosome as a sort of tetrad. From my own observations I can only say that the chromosomes are small irregular bodies, and it appears to me that, whenever chromosomes are concerned, Jörgensen's figures must be somewhat diagrammatic.

The formation of the first polar body in Grantia is represented in fig. 57, and it is evident that it takes place very much as described by Jörgensen for Sycon. The polar body

itself is seen to be of large size, and the remains of the spindle are seen as a very distinct cord, slightly thickened in the middle, connecting the group of chromosomes in the polar body with the group that remains behind in the oöcyte.

I have searched in vain for the second maturation spindle and second polar body, but, considering the rarity with which the first occurs in my specimens, their apparent absence has no significance. Even Jörgensen, however, was not able fully to observe the formation of the second polar body, though he tells us that apparently it also contains eight chromosomes, while eight (dyads ?) remain in the mature ovum.

(g) The Nurse-Cells and their Origin; Phagocytosis.

A process of feeding on the part of the growing oöcyte at the expense of certain nutrient cells has been described in the case of Sycon by both Görich (1903) and Jörgensen (1910). Görich says that the oöcytes ("Eizelle") ingest entire cells, which are probably themselves egg-cells of smaller size. The process is represented as taking place very much as in the case of an Amœba ingesting food-particles, except that no vacuole is formed around the ingested nutrient cell, whose protoplasm appears to mingle directly with that of the oöcyte. Each nutrient cell appears to be a small nucleated amœbocyte, with a relatively large, darkly staining particle (? chromidium) in the cytoplasm. Numerous very similar bodies, evidently chromidia, are figured in the cytoplasm of the feeding oöcyte, which appears to be at about the stage figured in my figs. 50 and 51 (compare Görich's figs. 1–6).

Jörgensen tells us that the ingestion of nutrient cells by the oöcyte takes place towards the end of the growth period, and goes on right up to the formation of the maturation spindle. The ingested cells are believed to be mostly oögonia and the ingestion is supposed to be due to chemotaxis. The ingested cells are said to be taken into a preformed gullet in

the oöcyte, and not, as described by Görich, by means of pseudopodia. Jörgensen has, however, observed ingestion by means of pseudopodia in the case of Sycon setosum. He also finds the presence of a compact chromidium in the cytoplasm to be characteristic of the nutrient cell. This chromidium may be taken into the gullet of the feeding oöcyte without the nutrient cell, but usually the entire nutrient cell is taken in. The chromidia received by the oöcyte in this way are distinguished by their size from those cast out from the nucleus, but both undergo like degeneration in the cytoplasm.

I have never seen anything resembling the formation of a gullet ("Schlund") in Grantia, and I think that Görich's account of the taking in of the nutrient cells in Sycon seems the more probable of the two.

In Grantia the process is complicated by the intervention of what I propose to term "nurse-cells," which capture the nutrient cells and bring them to the oöcyte. Possibly the supposed taking in by the oöcyte of the chromidium only from the nutrient cell really indicates something of the same kind for Sycon.

I have observed the feeding of the oöcyte by nurse-cells in specimens 11 and 23 and I have seen many instances of it, and I do not think there can be any doubt either as to the observations themselves or as to their interpretation. The peculiar arrangement shown in figs. 50 and 51 is frequently met with in my sections. I interpret it as indicating that a nurse-cell (*n. c.*) has captured a smaller cell which I regard as a nutrient cell or food-cell (*f. c.*), and is passing it into the cytoplasm of the oöcyte. I shall discuss the origin of the nurse-cell directly; in the meantime I may point out that it exhibits certain fairly well-marked characters of its own. Its cytoplasm is thin-looking and stains very lightly, and the nucleus is of moderate size, with a well-developed nuclear membrane and reticulum and a rather small nucleolus. The cytoplasm usually contains conspicuous inclusions which are evidently the remains of ingested and partially disintegrated cells, but nothing that can be identified with the "chromidium"

of the nutrient cells described by Görich and Jörgensen in Sycon; indeed, the nurse-cell does not appear to be itself a nutrient cell in the sense of these authors. The real nutrient cell (*f. c.*) is, however, a very conspicuous object, lying in the middle between the nurse-cell and the oöcyte. It appears to be always an oval or spherical cell with a reticulate nucleus and rather dense, finely granular cytoplasm, and it appears to be passed on to the oöcyte by the nurse-cell before it has undergone any disintegration, for its outlines are perfectly definite. Judging from its small size and the absence of chromidia in the cytoplasm I am inclined to think that the nutrient cell is probably a rounded-off collared cell, but it is impossible to be certain on this point, though I shall bring forward evidence presently to show that the nurse-cells do capture collared cells.

The process of feeding by nurse-cells does not appear to begin until the oöcyte has attained a considerable size, and it may be fed in this way both in the contracted and in the expanded condition, i. e. when the pseudopodia are reduced to short knobs and when they are fully extended. Figs. 50 and 51 represent the process of feeding by the nurse-cells as usually observed. A somewhat different condition is represented in fig. 52. The nurse-cell is here crushed in between the oöcyte, which is now much larger, and the layer of collared cells against which it lies. The nutrient cell has passed completely into the cytoplasm of the oöcyte, where it is surrounded by a small vacuole in which it appears to be undergoing disintegration, for instead of a distinct nucleus it exhibits two masses of chromatin—one at the middle and one at the side. Both nurse-cell and nutrient cell appear to be much smaller than in the cases previously described. The whole arrangement somewhat resembles the taking in of a chromidium from a nutrient cell as described by Jörgensen, but it is probably simply a more advanced stage of the feeding process shown in the preceding figures.

We come now to the question of the origin of the nurse-cells. I think there can be little doubt that they are derived

from small amœbocytes which occur scattered in the mesoglœa, such as is represented in fig. 58. It will be seen that we have here a cell of about the same size as the nurse-cell shown in fig. 51, and, except for the absence of cytoplasmic inclusions, closely resembling it. The cytoplasm is faintly staining and thin-looking, the nucleus is reticulate, with a small nucleolus and a well-developed nuclear membrane, and even the somewhat angular outline of the entire cell appears to be more or less characteristic, and is frequently met with again in the nurse-cells while in the act of feeding the oöcytes (compare figs. 50, 51). Whence these amœbocytes come it is impossible to say. They do not look like metamorphosed collared cells or epithelial cells, and they are very likely derived direct from embryonic amœbocytes. They probably do not all become nurse-cells, for some, which appear to be of the same nature, grow to a large size and may put out long filiform pseudopodia (figs. 59–61) In this extended condition I have found them both inside the flagellate chambers (fig. 60) and in the mesoglœa between them (fig. 61), while in a more or less rounded-off condition they are sometimes to be seen passing through the layer of collared cells, especially in the neighbourhood of the exhalant apertures of the chambers (fig. 59).

Such amœboid cells sometimes develop into very active phagocytes, apparently entirely on their own account. I have observed this in specimens 11, 22 and 24. Specimen 24 in particular is crowded with large and small phagocytes, which have evidently been feeding voraciously, and apparently chiefly on young germ-cells.

Fig. 62 represents a phagocyte from the mesoglœa, which has ingested a single relatively large cell, too large, I think, to be a spermatogonium, and so large that the cytoplasm of the phagocyte is only able to stretch itself around its prey in the form of a thin envelope. In fig. 63 an actively amœboid phagocyte, with filiform pseudopodia, is apparently in the act of ingesting a collared cell. Fig. 64 represents a phagocyte rounded off in the mesoglœa, with two partially disintegrated cells in its cytoplasm. This one looks as if it might have

become a nurse-cell, though rather large. Fig. 65 represents a very actively amœboid phagocyte, while fig. 66 shows one squeezing itself through the layer of collared cells. Fig. 67 shows one with root-like pseudopodia, half in and half out of a chamber; this one has collected and ingested no less than six cells, which I judge from their size and appearance to be spermatogonia. Fig. 68 represents yet another hanging on to the inner surface of the layer of collared cells.

It appears, then, that while some of these amœbocytes exercise their phagocytic propensities in favour of the oöcytes, and become nurse-cells, others feed voraciously on their own account, even entering the flagellate chambers and apparently collecting the numerous young germ-cells that are found therein. It seems not improbable that this excessive phagocytosis may be regarded as a perverted instinct, for the phagocytes appear to take in food far beyond their own possible requirements, and yet I have never seen a large phagocyte feeding an oöcyte. It is perhaps worth noting that specimen 24, in which most of the cases of phagocytosis by large amœbocytes were observed, had been kept in the circulation for a week before being killed and preserved. The abnormal conditions may have stimulated the amœbocytes to an abnormal activity in phagocytosis.

The possibility also occurs to one that some of the large phagocytes are parasitic Amœbæ which do not belong to the sponge at all. Mr. Orton (1913) has recently described Amœbæ from the gastral cavity of Sycon which certainly seem to be quite independent organisms, though I at first thought otherwise (Dendy, 1913), and it seems by no means improbable that such Amœbæ may enter the chambers and feed upon the young germ-cells, or even force their way into the mesoglœa. The chief argument against this view appears to be the impossibility of distinguishing between the larger and smaller phagocytes, and the apparent identity of the latter with the nurse-cells, which must certainly be regarded as belonging to the sponge itself.

(h) Summary and General Remarks on Oögenesis.

It will be seen from the foregoing account of my observations on the oögenesis of Grantia, that in their main features they agree with what has already been recorded, especially by Jörgensen, for the closely related genus Sycon. In some important respects, however, and especially as regards the derivation of the oögonia from collared cells, my observations differ from those of Jörgensen, and, while I have not been able to obtain anything like such precise results as he claims with regard to the mitotic phenomena, I have been able to describe a great deal that has either escaped his notice or does not occur in Sycon, concerning, for example, the feeding of the young oöcytes in the chambers and the subsequent formation of chromidia by extrusion of nucleolar matter into the cytoplasm, the very remarkable feeding of the oöcytes by means of nurse-cells, and the process of phagocytosis in general.

The process of oögenesis in Grantia may be briefly summarised as follows :

The primary oögonia are directly derived from collared cells, which accumulate reserve material, enlarge, withdraw their collars and flagella, become amœboid and wander into the mesoglœa, re-entering the chambers before dividing mitotically into the oögonia of the second generation. Prior to this division the nucleolus appears to be bodily cast out of the oögonium.

The oögonia of the second generation divide again while in the chambers, and probably almost immediately, into small oöcytes.

The small oöcytes become amœboid, and, while still within the chambers, attached by pseudopodia to the layer of collared cells, take in food-particles and form conspicuous chromidia in their cytoplasm. Having increased considerably in size they leave the chambers and enter the mesoglœa.

Here they continue to undergo a process of extensive chromidium-formation by extrusion of chromatin from the nucleolus into the cytoplasm.

They also probably undergo about this time the prophases of the first maturation division.

They now send out anchoring pseudopodia by which they became attached to the layer of collared cells and probably draw nutriment from them.

Presently they undergo a remarkable contraction, the pseudopodia being almost completely withdrawn, and about this time they begin to be fed by special nurse-cells, which collect smaller cells and pass them into the growing oöcyte.

The oöcyte increases greatly in size, and long root-like pseudopodia are again put out in a plane parallel to the layer of collared cells against which it lies.

The nucleus becomes very large and vesicular, with a huge spherical nucleolus, and chromidia are abundantly formed in the cytoplasm, though apparently no longer directly derived from the nucleolus, but formed probably by extrusion of granules of chromatin from the nucleus. The chromidia (yolk-nucleus), whatever their source, are probably concerned in the elaboration of the deutoplasm, with which the cytoplasm becomes uniformly and densely charged, though definite individual yolk-granules can hardly be recognised.

When the oöcyte has reached its full size it withdraws its pseudopodia and rounds itself off, the nuclear membrane disappears and the contents of the nucleus disperse themselves through the cytoplasm. The nucleolus remains recognisable for some time after this event, but gradually becomes absorbed.

Chromosomes have not been recognisable since the oögonial mitoses, when they appeared in the equatorial plate as a group of eight or ten minute, irregularly rounded bodies, each of which presumably divided into two. They now appear again on the first maturation spindle, but only one really good spindle was found, and that already in the anaphase.

The first polar body is formed in apparently a typical manner, exactly as described by Jörgensen for Sycon, and is of large size.

The second maturation spindle and second polar body,

described by Jörgensen for Sycon, were not met with, though they probably occur.

No evidence was obtained of a reducing division, and, indeed, the number of chromosomes could never be accurately counted.

In spite of the fact that we have no reliable information with regard to the phenomena of meiosis in sponges, we cannot fail to be struck with the close general agreement of the process of oögenesis with the same process as observed in higher animals. The multiplication of oögonia; the formation of chromidia or yolk-nucleus by the oöcyte; the early inception of the first maturation division (if this be confirmed), interrupted by the long period of growth; the co-operation of other cells in the process of nutrition; the character of the nucleus and the formation of the polar bodies; are all features which the sponges share with higher groups, and one can hardly avoid asking the question, Does this close similarity in oögenesis point to a nearer relationship of the sponges with the Enterozoa than is usually admitted in this country? The question is well worthy of consideration, but we can hardly hope for a final solution of it in the present state of our knowledge. We can hardly abandon the choano-flagellate ancestry of the sponges without much stronger evidence than we possess, and we certainly are not justified in attributing a choano-flagellate ancestry to other groups of Metazoa. May we, then, suppose that all the essential processes of oögenesis already existed in pre-choano-flagellate Protozoon ancestors common to sponges and Enterozoa? Such a supposition would certainly be in harmony with the view now generally held that the germ-cells of the higher animals are really equivalent to so many Protozoa, for if this be so then the phenomena of oögenesis must be such as we might reasonably expect to find in Protozoa. Recent advances in protozoology, I think, show that such an expectation is likely to be fulfilled, for we already know that the gametes themselves may attain as high a degree of differentiation (into ovum and spermatozoon) in Protozoa as in Metazoa. We also know that the female gamete may accumulate yolk (e. g. in Coccidium), and that

something that may reasonably be interpreted as a formation of polar bodies may take place (e. g. Paramœcium). I venture to predict that a good deal of light will, in the near future, be thrown upon the complex phenomena exhibited in the life-history of many Protozoa, by comparison of the events that take place in the gametogenesis of higher animals. Some more satisfactory and uniform system of terminology will, however, have to be evolved before much progress can be made in this direction. We shall have to know, for example, exactly what we mean by "chromidia." Prof. Minchin (1912) tells us that "in a great many Sarcodina, especially in those belonging to the orders Amœbæa and Foraminifera, chromidia may be present in the gamete-forming individuals as a permanent constituent of the body-structure. In such cases the chromidia represent, wholly or in part, the generative chromatin, and give rise by formation of secondary nuclei to the nuclei of the gametes." In the present paper I have, following Jörgensen, used the term "chromidia" for all the chromatin which occurs in the cytoplasm. This, I think, is probably all extruded from the nucleus (and nucleolus), and is almost certainly concerned in yolk-formation, and therefore "trophochromatin" and not "idiochromatin." The difficulty of distinguishing between these two kinds of "chromatin" forms perhaps the chief obstacle in the way of further progress in the direction indicated.

(g) Spermatogenesis.

(a) Historical.

I have already referred to Haeckel's discovery of the sperm-morulæ of calcareous sponges in the gastral epithelium of the flagellate chambers, and to his opinion that they arise by division of collared cells. These observations never met with general acceptance, and are usually regarded as having been superseded by Poléjaeff's well-known work, 'Über das Sperma und die Spermatogenese bei Sycandra raphanus Haeckel' (1882).

Poléjaeff lays stress upon the discrepancies between the account of the spermatozoa given by Haeckel and that given by Eimer. According to Eimer, the spermatozoa, if not isolated, occur scattered through the tissues, united in millions in oval balls; according to Haeckel, they lie between the collared cells with their tails projecting freely into the cavity of the flagellate chamber, and it is never possible to find them in considerable quantities. According to Eimer, again, they are to be distinguished from the collared cells by the character of the movements of the flagella; while, according to Haeckel, these movements show no essential differences in the two cases. I think it almost certain myself that, although Haeckel saw the sperm-morulæ in the situation he describes, he did not see the tails of the spermatozoa, for the sperm-morulæ represent a comparatively early stage of spermatogenesis at which no tails have yet appeared. Haeckel probably mistook for spermatozoon tails some of the flagella of the collared cells amongst which the sperm-morulæ lie. The discrepancy as to numbers and position is not a very serious matter. Poléjaeff himself has shown how enormously the number of spermatozoa produced differs in different individuals, and I find myself that in Grantia, although the sperm-morulæ are generally to be observed in the walls of the flagellate chambers, or lying free in the chamber-cavities, some of the early stages of spermatogenesis occur in the mesoglœa, while later stages are found in the inhalant canals (possibly of different individuals), and these might well appear in sections to be lying in the tissues.

Poléjaeff (1882) found that in Sycon raphanus, although the sponge is hermaphrodite, the vast majority of individuals are predominantly female, and only very occasionally a predominantly male specimen is forthcoming. In the latter, however, the sperm-balls ("Spermaklumpen") were so numerous that their whole development could be traced in a single section. He derives these sperm-balls from ordinary amœbocytes ("Wanderzellen") in the mesoglœa (mesoderm), similar to those which, in his opinion, give rise to the ova. These

cells have a diameter of 0·008–0·02 mm., and their bright, vesicular nuclei are distinguished by their relatively large size and their highly refractive nucleolus. Such a cell is represented in Poléjaeff's fig. 3*a* as a spherical body with an excentrically placed nucleus. The nucleus now divides into two somewhat unequal parts, which take up their positions at opposite poles of the cell, which becomes differentiated into two corresponding parts, a cover-cell and a sperm mother-cell ("Ursamenzelle"). The cover-cell does not divide again, but the nucleus of the sperm mother-cell divides repeatedly, and finally gives rise to a large number of very minute, granule-like spermatozoon-heads, enclosed within a capsule formed by the cover-cell. Each of these heads presently becomes provided with a cytoplasmic tail. During this process there is no increase in volume of the sperm-ball, and Poléjaeff remarks upon the extraordinarily small size of the spermatozoa. He also points out that the spermatogenesis of Sycon as described by him differs in several respects from that described for non-calcareous sponges by Schulze, Keller and others. Thus the division of the nucleus of the "Ursamenzelle" is not immediately followed by division of the cytoplasm, so that there arises a multinucleate mother-cell and not a true sperm-morula, but he remarks that this is not a matter of any very great importance. More significant, perhaps, is the absence of the endothelial capsule formed around the sperm-ball by the mesoglœa cells in some of the non-calcarea. This, I think, is a matter of some importance in connection with the problem of how the spermatozoa are transferred from one sponge to another. This problem Poléjaeff does not attempt to solve, and, indeed, we are left in doubt, after studying his paper, as to whether or not he considers that self-fertilisation takes place in Sycon. His fig. 1 shows immense quantities of what appear to be spermatozoon heads in the inhalant canals, the chambers and the exhalant canals, as well as sperm-balls in the mesoglœa, but no attempt is made to decide the question whether or not all this mass of sperm has been derived from the same sponge.

I think the investigation of this question would probably have gone a long way towards reconciling the discrepancies between the observations of Haeckel and those of Eimer, and have shown that the cover-cells retain their wandering propensities for some time, and carry the spermatgonia from the mesoglœa into the collared-cell layer, whence they are discharged into the water-stream and carried out of the sponge altogether, possibly to find their way back again, either into the same or into another sponge, through the inhalant canal-system. My own observations clearly indicate that this is the course of events, though there appear to be noteworthy differences in details of behaviour between the two genera Sycon and Grantia.

During the thirty-two years that have passed since the publication of Poléjaeff's memoir, the only contribution that has been made to the very difficult problem of the spermatogenesis in calcareous sponges is that contained in the paper by Wilhelm Görich—"Zur Kenntnis der Spermatogenese bei den Poriferen und Cölenteraten nebst Bemerkungen über die Oogenese der erstern" (1903). This author again deals with the process as exhibited in Sycon raphanus, and although he describes only a few of the earlier stages in this sponge, his results are in one respect strikingly at variance with those of Poléjaeff. He agrees with the latter in deriving the spermatogonia from mesoglœal amœbocytes, which round themselves off at an early stage of their growth as compared with the oögonia. He also describes the formation of a cover-cell, but in a totally different manner from that described by Poléjaeff. The spermatogonium and the cover-cell, though both derived from amœbocytes of the mesoglœa, differ from one another in certain particulars and do not arise by division of a common mother-cell. They only come into relation with one another secondarily, the cover-cell spreading itself around the spermatogonium, and finally ingesting it, in a way which is evidently very similar to the process of phagocytosis already described by me for Grantia. The result, however, is a spermatogonium enclosed in a mother-cell very much as

described by Poléjaeff. The spermatogonium is represented as dividing mitotically into two and then incompletely into four parts, beyond which its history was not followed.

This is all that is known of the spermatogenesis in Calcarea. Amongst other sponges the most frequently and most fully investigated form is Spongilla, and it is perhaps worth while to say a few words about what is known in this case, which seems to be typical of the non-Calcarea, before proceeding to describe my own observations on Grantia.

As far back as 1888, Fiedler published his memoir, "Über Ei- und Spermabildung bei Spongilla fluviatilis." He describes the formation of cover-cell and sperm mother-cell ("Spermatocyte") by division of a common mother-cell ("Spermatogonium") exactly as described by Poléjaeff for Sycon. The cover-cell, however (of which more than one may be found), not infrequently disappears before the contained "spermatocytes" have completed their development, and the mass of sperm, which may have been derived from several "spermatogonia," becomes enclosed in a secondary follicle formed from ordinary mesoglœal cells ("Parenchymzellen"). The original "spermatocyte" divides repeatedly by mitosis into daughter-cells which are completely separated from one another. The smallness of the objects, however, makes the examination of the process very difficult and the details of mitosis are not very satisfactorily given. The last generation of spermatocytes, the spermatids, develop directly into the spermatozoa. A compact chromatin-ball is formed by contraction of the nucleus, as previously described by Schulze for Halisarca (= Oscarella) (1877) and Aplysilla (1878), and the enveloping cytoplasm is drawn out into a slender tail. In the fully formed spermatozoon the chromatin-ball forms a minute spherical head.

Görich (1903) added some interesting particulars as to Spongilla, especially with regard to the structure of the fully formed spermatozoon, in the paper which I have already quoted. He finds that the number of cover-cells taking part in the formation of the capsule or spermatocyst varies from one

to about six, and maintains that these cover-cells are derived from mesoglœal cells distinct from the spermatogonium as in the case of Sycon. He brings forward very little evidence, however, in support of this view. He describes the often repeated mitotic division of the sperm-cells within the spermatocyst very much as it was described by Fiedler. In the fully developed spermatozoa, however, he finds a far more complex structure than had been observed by any of his predecessors, for in addition to the spherical head and long slender tail he describes and figures middle piece, apical body and centrosomes, thus bringing the structure closely into line with that of the spermatozoon in Enterozoa, as exemplified by Aurelia, which he describes and figures in the same paper.

With regard to the explanation of the close resemblance thus established between the spermatogenesis of sponges and that of the Enterozoa, and its bearing upon the relationship of the two groups, I may refer to what I have already said in my summary on the oögenesis.

(b) Origin and Growth of the Primary Spermatogonia.

In returning to Haeckel's view that the spermatozoa are formed by division of collared cells, I must admit that it is extremely difficult to bring forward convincing evidence that this is really the case. Haeckel, of course, was of opinion that the collared cells become divided up into spermatozoa in sitû, and he says nothing of the existence of the spermatocyst or cover-cell described by Poléjaeff and Görich, while both the latter hold that the spermatozoa develop from amœbocytes of the mesoglœa. I believe that the view of each of these authors expresses part of the truth, and I hope that my own observations may serve to account for, and to a large extent to reconcile, the discrepancies between them. The manner in which I have interpreted these observations and arranged the different stages cannot even yet, however, be regarded as more than tentative.

The first stage in spermatogenesis, as in that of oögenesis, appears to be the enlargement of individual collared cells in the lining epithelium of the chambers (figs. 69, 70). At the same time the cytoplasm acquires a peculiar curdled appearance (if I may use this expression for want of a better), which looks as if it might be due to the running together of the reserve granules. Irregular inclusions of large size may thus be formed, around which vacuoles frequently make their appearance. By these appearances it is possible to distinguish between what I believe to be the primary spermatogonia and the primary oögonia respectively, for in the latter (figs. 11, 12) it will be remembered that the reserve granules remain separate and do not run together in irregular masses.

The nucleus now becomes very distinctly reticulate as compared with that of neighbouring collared cells (figs. 71, 72), collar and flagellum are withdrawn, and the cell puts out pseudopodia and becomes amœboid (figs. 72–74). In this condition the primary spermatogonia are to be found hanging into the chamber from the layer of collared cells by means of their pseudopodia. Definite inclusions disappear from the cytoplasm.

The cell now rounds itself off and assumes a very characteristic appearance (figs. 75, 76). It is readily distinguished from the oögonia by its smaller size and by the character of the nucleus. It is also quite different in character from the young oöcytes, which are of about the same size (figs. 29, 30), especially as regards the nucleus, which is coarsely reticulate and without a really well-defined nucleolus, while that of the young oöcyte has a very conspicuous nucleolus surrounded by a clear zone and then by a zone of fine granules.

I have found the primary spermatogonia in their rounded-off condition in the mesoglœa as well as in the flagellate chambers, so that it seems probable that while still in the amœboid state they may migrate through the chamber-walls as the oögonia and oöcytes so frequently do.

It is interesting to observe that the primary spermatogonia exhibit a good deal of variation in size, as is shown in fig.

75. In one case also (fig. 75 a) I have observed mitosis in the free spermatogonium, but I have no evidence that the spermatogonium ever actually divides until it has been provided with a cover-cell.

(c) Formation of the Spermatocysts or Cover-cells.

It will be remembered that according to Poléjaeff the original amœbocyte in Sycon divides into two parts, one of which forms the cover-cell and the other the primary spermatogonium, while Görich claims that the cover-cell is formed by an independent amœbocyte which approaches and envelopes the spermatogonium in the mesoglœa. My own observations strongly support the latter view, and I regard the process of envelopment of the spermatogonium by the cover-cell as a special case of phagocytosis. Indeed, as already pointed out, spermatogonia are frequently ingested by the phagocytes, and it is difficult, if not impossible, to distinguish an ordinary case of phagocytosis in which only a single spermatogonium has been ingested, from a case of cover-cell formation (cf. fig. 62, in which the ingested cell, however, seems too large to be a spermatogonium). Figs. 77, 80 and 81 represent spermatocysts, with enclosed primary spermatogonia, lying in the mesoglœa. In these cases the cover-cell appears to resemble closely a small phagocyte such as gives rise to the nurse-cells (cf. fig. 58).

The majority of the spermatocysts, however, are found lying in the walls of the flagellate chambers between the collared cells, with the enclosed spermatogonium either still undivided, as shown in figs. 78 and 79, or in process of division, as shown in fig. 82, or, much more frequently, divided up into a sperm-morula (figs. 84, 85). When in this position the spermatocyst certainly looks very much as if it were derived in sitû from a collared cell. Sometimes, it is true, the nucleus is distinguishable from that of adjacent collared cells by its reticulate character (figs. 82, 85), but in other cases (figs. 78, 84) no such distinction can be made out.

I have already pointed out, however, that the nuclei of the collared cells themselves vary very greatly in appearance, and that reticulate and uniformly dark-stained nuclei may occur in adjacent cells. Sometimes the cytoplasm of the cover-cell may even contain reserve granules like those found in the collared cells (fig. 85).

The history of the spermatogonia themselves, however, and the phagocytosis observed in the mesoglœa, seem to me to indicate very clearly that the spermatocysts so often seen lying between the collared cells have reached their position by migration, and there is no sufficient reason for concluding that the cover-cells are ever derived from collared cells.

As the spermatocyst lies in the collared cell layer its nucleus is situate, usually, at any rate, towards the lumen of the chamber, just as are the nuclei of the collared cells themselves, and the spermatogonium or sperm-morula is enclosed in its basal portion (figs. 78, 82, 84).

As the sperm-morula develops the cyst formed by the cover-cell becomes extremely thin (fig. 87) and finally ruptures, discharging the sperm-morula into the cavity of the chamber (fig. 83). Thus the liberation of the sperm-morula from the cover-cell takes place much earlier than in the case of Sycon, where, according to Poléjaeff, spermatozoa are formed while still within the cysts.

(d) Development of the Sperm-morulæ from the Primary Spermatogonia.

The first division of the primary spermatogonium at least appears to take place mitotically, as has already been described by Görich for Sycon. At any rate one sometimes finds spermatogonia in which the nucleus has disappeared, and what appear to be small, scattered chromosomes, eight or ten in number, are scattered through the cytoplasm as shown in figs. 81 and 82, while fig. 80 represents what may be a spireme stage.

I have only once observed what appears to be the two-

celled stage of the sperm-morula (fig. 83), and I regard this as a somewhat doubtful case; it shows, however, two distinct spherical bodies, which I take to be secondary spermatogonia, enclosed in what is presumably a cover-cell (*cov.*) lying in the layer of collared cells.

The four-celled stage I have never been able to find, in spite of prolonged searching. The eight-celled stage, however, I have seen several times (figs. 84, 85, 86), and it appears that the sperm-morula may be liberated from the spermatocyst as early as this (fig. 86).

A stage in which the morula consists of sixteen cells, or thereabouts, is the most frequent in my preparations. This stage sometimes occurs still enclosed in the cover-cell (fig. 87), but more frequently lying free in the cavity of the flagellate chamber (figs. 88–92). A remarkable feature of the eight-celled and sixteen-celled stages is the extraordinary distinctness with which the daughter-spermatogonia are defined, but the exact appearance evidently depends somewhat upon the method of preparation. Very often they appear as little heaps of highly refractive spherical balls, like small shot, stained black or nearly so (figs. 83, 88). At other times they are much more lightly stained, and one or more minute, more darkly stained granules appear within them (figs. 86, 87, 89, 90). How the division takes place I cannot say, but I have seen no sign of mitosis after the first division of the primary spermatogonium. Whether or not the spherical bodies represent entire cells, or nuclei only, I am also unable to say positively, but I conclude from their general appearance that the former is the case. It will be noticed that there is considerable difference in size between the spermatogonia in different sperm-morulæ of apparently the same stage of development (cf. figs. 87, 88), but this is only what might be expected from the differences in size of the primary spermatogonia already mentioned. Although usually spherical, the daughter-spermatogonia sometimes appear to be polygonal from mutual pressure (figs. 89, 91).

A noteworthy feature is the appearance between them in

the sperm-morula of a substance that looks like residual protoplasm (figs. 86–92). As development proceeds (at the sixteen-celled stage), this material seems to swell up so as to separate the spermatogonia more or less from one another.

(e) The Formation of Spermatozoa; Comparison with Sycon, etc.

As to how the spermatozoa are developed from the sperm-morulæ in Grantia I have no definite information to offer. My material does not suffice to settle this question, but I have some reason to believe that the spermatogonia of the sixteen-celled stage undergo further repeated subdivision. I believe that this usually takes place after the sperm-morulæ have been transferred by the water currents to the inhalant canals of another individual. I have occasionally found, both in the inhalant canals and flagellate chambers, small masses of darkly stained granules (fig. 93) which appear to be identical with the masses of granules which Poléjaeff showed to be spermatozoon heads in Sycon. If they be of this nature, as I think highly probable, their minute size certainly serves to indicate a further breaking up of the spermatogonia of the sixteen-celled stage. I have also a small amount of direct evidence of such breaking up of the sperm-morulæ in the inhalant canals, but it is not conclusive enough to bring forward definitely.

Poléjaeff tells us that in Sycon the spermatozoa are formed by repeated subdivision of the spermatogonia within the cover-cell, during which process the spermatocyst does not increase in size. The final products of these divisions are represented as being extremely minute, and each becomes provided with a slender flagellum.

I happen to have in my possession one of Poléjaeff's own preparations of Sycon, sent by him to Mr. Carter in 1883. This preparation shows "sperm-balls" (sperm-morulæ) in the inhalant canals, and I suppose them to have come from another individual. The sperm-balls vary considerably in

size (fig. 94) and each is still enclosed in the cover-cell, whose nucleus is very distinctly visible. In the interior a number of ill-defined, faintly stained bodies are present, which may be daughter-spermatogonia, or, as Poléjaeff supposes, merely the nuclei of these. They are nothing like so definite as the spermatogonia which I find in the sperm-morulæ of Grantia, but this may be partly because they belong to a later stage of development, and partly because of differences in the mode of preparation. According to the information given in his memoir Poléjaeff's preparations were made with osmic acid (0·01–0·05 per cent.) material stained with alum carmine, and this probably applies to the preparation in my possession.

I give in fig. 94 drawings of two sperm-balls from this preparation. It will be seen that they are of just about the same size as the spermatocysts with enclosed primary spermatogonia in Grantia (cf. figs. 77, 78, 81), and, allowing for the longer retention of the cover-cell and the further subdivision of the spermatogonia after the sixteen-celled stage, I think there is no serious discrepancy. I am unable to make a direct comparison between the earlier stages in the two genera as I have found none of these in Poléjaeff's preparations.

That the sperm-morulæ found by me in Grantia are identical with the structures already referred to as described by Haeckel in the lining epithelium of the flagellate chambers of various calcareous sponges, appears to me to admit of very little doubt, although of course I cannot agree with him in the details of his account.

It has occurred to me as just possible that someone may suggest that these bodies are not sperm-mornlæ at all, but of quite a different nature; that they may be Sporozoa living for a period as intracellular parasites in the collared cells and possibly also in the amœbocytes of the sponge. Apart from the fact, however, that sporozoan parasites have never yet been observed in sponges, I think the developmental history of the bodies in question is sufficient to negative this view. No infection of the sponge-cells by small forms that might be young Sporozoa has ever been observed, and the cell which

is surrounded by the cover-cell, and which I believe to be the primary spermatogonium, has nothing about it to suggest a sporozoon.

I can only regret that I am unable to give a more satisfactory account of the spermatogenesis, but I hope that what I have said will arouse more interest in this extremely difficult problem, and enable future workers at any rate easily to find the structures in question, and perhaps fill up the many gaps which I have left.

(h) Fertilisation of the Ovum.

I have not been fortunate enough to observe the actual entrance of the spermatozoon into the mature ovum. According to Jörgensen (in Sycon), the head swells up and gives rise to the male pronucleus, in which a nucleolus very soon makes its appearance. The female pronucleus has in the meantime developed from the group of chromosomes that remained behind in the ovum after the formation of the second polar body. These unite together into a compact chromatin ball, around which a vacuole, enclosed in a nuclear membrane, makes its appearance. A nucleolus is very early differentiated in the midst of this mass, the remainder of which is broken up into chromatin granules, which become scattered over the nuclear reticulum.

Sometimes a portion of one of the pronuclei (either male or female) is represented by a small separate "karyomere," so that there appear to be three nuclei in the fertilised egg. The number of nucleoli present in the pronuclei varies.

So far as they go my own observations on Grantia are entirely in harmony with these results. I have been able to study four ova with well-developed male and female pronuclei. In only one of these cases were the two pronuclei both single, and in this case the number of nucleoli was either 4 + 1 or 3 + 2, one of them having been displaced in the cutting. In the other three cases a karyomere was present in addition to the principal pronuclei, and the numbers and distribution of

the nucleoli were 1 + 1 + 1, 1 + 1 + 2, and 1 + 1 + 2 respectively.

Fig. 95 shows one of these cases. The position of the degenerating polar body (*p. b.*) shows that the large pronucleus with the single large nucleolus is evidently the principal female pronucleus, and the small karyomere, with single nucleolus, doubtless belongs to it. The male pronucleus, on the right and below, has two nucleoli.

The formation of karyomeres is a very curious and striking phenomenon, for further details as to which I may refer the reader to Jörgensen's paper. That author points out that karyomere formation also takes place in Sycon in the process of segmentation of the fertilised ovum, and I find the same to be true in the case of Grantia.

It is perhaps worth while pointing out that the nucleoli in the male and female pronuclei do not stain nearly so deeply as the nucleoli of the young oöcytes. The same is also true of the nucleoli in the older oöcytes. The difference may perhaps be correlated with the fact that in the young oöcytes the nucleolus is actively engaged in the formation of chromidia or "yolk-nuclei," while probably it is not directly engaged in this process in the older oöcytes, and certainly not in the fertilised ovum, where yolk-formation has ceased.

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(L) EXPLANATION OF PLATES 23 TO 26,

Illustrating Prof. Arthur Dendy's paper, "Observations on the Gametogenesis of Grantia compressa.

[All figures are magnified about 1650 diameters, and, with the exception of fig. 94, refer to Grantia compressa.]

PLATE 23.

Fig. 1.—Collared cell putting out pseudopodia, from teased preparation of living sponge. *col.* Collar. (Unstained.)

Fig. 2.—Collared cell (*a*), with flagellum still present, putting out pseudopodia and retreating into the mesoglœa from the gastral epithelium. *b.*, *b.* Collared cells still in position in the gastral epithelium. (Specimen 24. Borax carmine.)

Fig. 3.—Amœbocyte (*a*) lying behind the gastral epithelium and probably derived from a collared cell. *b.*, *b.* Collared cells. (Specimen 24. Borax carmine.)

Fig. 4.—Portion of section at right angles to the gastral surface, showing granular epithelial cell (*a*) in the "flask-shaped" condition at the surface, and amœbocyte (*b*) in the mesoglœa. *sp.* Spicules. (Specimen 24. Iron brazilin.)

Fig. 5.—Amœbocyte in the mesoglœa just beneath the gastral cortex. (Specimen 24. Iron brazilin.)

Fig. 6.—Portion of section at right angles to the gastral cortex, showing parts of two granular epithelial cells (*a*) in position on the surface, and a group of amœbocytes (*b*) lying in the mesoglœa and evidently derived by immigration from the epithelial layer. *p. g.* A mass of pigment-granules apparently discharged from an amœbocyte. (Specimen 24. Iron brazilin.)

Fig. 7.—Portion of section through inhalant canal (*i. c.*) and adjacent mesoglœa, showing transition from epithelial to amœboid cells. (Specimen 24. Iron brazilin.)